INFECTION AND IMMUNITY, Dec. 1985, p. 900–904
0019-9567/85/120900-05$02.00/0

Vol. 50, No. 3

Monoclonal Antibodies That Recognize the P Fimbriae $F7_1$, $F7_2$, F9, and F11 from Uropathogenic *Escherichia coli*

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Received 20 May 1985/Accepted 30 August 1985

The *Escherichia coli* P fimbriae $F7_1$, $F7_2$, F9, and F11 from four cloned strains were purified, and polyclonal antisera were raised in rabbits. Cross-reactions of these antisera with eight different cloned and purified fimbriae were measured in an enzyme-linked immunosorbent assay. These antisera showed a reaction with the homologous fimbriae and also with most heterologous fimbriae. Monoclonal antibodies (MAbs) directed against the same four native fimbriae were produced by the fusion of spleen cells from immunized BALB/c mice with SP2/0 myeloma cells. The resulting four series of MAbs were also screened in an enzyme-linked immunosorbent assay with eight different cloned and purified fimbriae. Four different $F7_1$ hybridomas produced MAbs which recognized only epitopes on $F7_1$ fimbriae. Two $F7_2$ MAbs recognized epitopes on $F7_2$ and F9 fimbriae, whereas another $F7_2$ MAb recognized an epitope on only $F7_2$ fimbriae. Three MAbs raised against F9 reacted only with epitopes on F9 fimbriae. Six MAbs against F11 fimbriae could be divided into two groups: on the one hand two MAbs recognizing F11, pyelonephritis-associated pilus, Pap, and $F7_2$ fimbriae and on the other hand four MAbs recognizing F11 and "Clegg" fimbriae. None of the MAbs reacted with 1A or 1C fimbriae. In a hemagglutination inhibition assay it was shown that none of the MAbs produced inhibited the adhesive properties of homologous cloned strains.

One of the most important virulence factors of uropathogenic *Escherichia coli* is the capacity of these bacteria to adhere to urepithelial cells (6, 22, 26). Adhesion is mediated by fimbriae which are heterogenous serologically (18, 19, 20) and with regard to the molecular weights of their subunit proteins (8). Ørskov and Ørskov (18) distinguished eight serologically different fimbriae in uropathogenic *E. coli*, and Parry et al. (20) found seven serologically different fimbriae. Fimbriae from uropathogenic *E. coli* can also be distinguished by their receptor specificities: (i) fimbriae that cause mannose-sensitive hemagglutination of guinea pig erythrocytes (type 1 fimbriae); (ii) fimbriae that cause no hemagglutination (HA) of any erythrocytes (e.g., 1C fimbriae); and (iii) fimbriae that cause mannose-resistant hemagglutination (MRHA) of human erythrocytes. This last group can be subdivided on the basis of receptor specificities into P, M, S, and X fimbriae (9, 12, 24). The P fimbriae especially have been associated with the pathogenesis of pyelonephritis (23). Recently, the molecular cloning of several fimbriae was reported. Hull et al. (7) and Clegg (2) described the molecular cloning of pyelonephritis-associated pilus (Pap) fimbriae and "Clegg" fimbriae, respectively, both serologically not determined but not cross-reactive with each other (2). The molecular cloning of the genes coding for $F7_1$ (29), $F7_2$ (28), and 1C (27a) fimbriae from one parental strain was recently reported. The fimbriae cloned by Rhen et al. (21) are presumably the same as the $F7_1$, $F7_2$, and 1C fimbriae, but they were cloned from another parental strain. We have recently reported the molecular cloning of F9 (3) and F11 (3a) fimbriae, and Hacker et al. (5) described the cloning of X fimbriae from a uropathogenic *E. coli* strain. Orndorff and Falkow (17) cloned type 1 fimbriae from a clinical isolate of *E. coli*, and Klemm et al. (9a) cloned type 1 fimbriae from an *E. coli* K-12 strain.

In the present study, we describe the production and characterization of polyclonal and monoclonal antisera against four different cloned P fimbriae: $F7_1$, $F7_2$, F9, and F11.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth media. AM1727 is a *recA* derivative of JE2571, an *E. coli* K-12 strain deficient in the production of type 1 fimbriae (28). All fimbriae used in this study were purified from AM1727 harboring the clones described in Table 1. The $F7_1$, $F7_2$, and 1C clones were kindly provided by Irma van Die, the Pap clone was provided by Staffan Normark, and pDC1 was provided by Steven Clegg.

All bacteria were cultivated on brain heart infusion agar (Oxoid Ltd.), except that strain AM1727 harboring pPIL38 was grown in brain heart infusion broth. Selective pressure against the loss of plasmids was imposed by adding 50 μg of ampicillin, 100 μg of chloramphenicol, or 12.5 μg of tetracycline per ml, dependent on the cloning vector used.

Purification of fimbriae. Fimbriae were purified essentially by the method of Korhonen et al. (10) with some modifications, yielding purified native fimbriae as described previously (3). Purity was checked by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis by the method of Lugtenberg et al. (15). Fimbria concentrations were measured by the method of Lowry et al. (14).

Polyclonal antisera. Rabbits were inoculated intramusculary on days 0, 9, and 18 with 400 μg of purified fimbriae in Freund complete adjuvant (Difco Laboratories). Serum was collected 5 days after the last injection.

Hybridoma production. For immunization, BALB/c mice were inoculated intravenously via the tail vein with 10 μg of purified fimbriae in 0.5 ml of saline on days 0, 5 and 10. Four days after a booster injection with the same amount of fimbriae, the mice were killed, and 4×10^7 spleen cells were combined with 2×10^7 cells of the nonproducing myeloma cell line SP2/O. Spleen cells and myeloma cells were fused by using polyethylene glycol (PEG 4000) and suspended in

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TABLE 1. Plasmids encoding different fimbriae

Plasmid	Fimbriae	Mol wt	HA	Reference(s)
pPIL110-70	$F7_1$	22,000	MRHA	29
pPIL110-37	$F7_2$	19,160	MRHA	28
pPIL288-10	F9	21,000	MRHA	3
pPIL291-15	F11	18,000	MRHA	3a
pRHU845	Pap	19,500	MRHA	1, 7
pDC1	Clegg	17,000	MRHA	2
pPIL110-51	1C	15,800	None	27a
pPIL38	1A	15,706	Mannose-sensitive Ha	9a

complete growth medium (Dulbecco modified Eagle medium; Flow Laboratories, Inc.) containing 1% L-glutamine, 1% pyruvate (Sigma Chemical Co.), nonessential amino acids (Flow), 15% fetal calf serum (GIBCO Laboratories), 2% hypoxanthine (Merck & Co., Inc.), 2% desoxythymine (Merck), and 1% aminopterin (Sigma). After being seeded in four microtiter plates (96 wells), the cells were grown in a 5% CO_2 atmosphere at 37°C and 95% humidity. After 3 weeks of incubation, all wells were tested for antibody production against fimbriae with an enzyme-linked immunosorbent assay (ELISA). Positive clones were transformed to 24-well plates for additional growth, followed by a limiting dilution step to obtain cell lines derived from one single hybrid cell. After additional growth, the cells were used for antibody production in ascitic fluid.

Production of ascitic fluid. Pristane-primed BALB/c mice were irradiated with 450 rads. One day after irradiation, the mice were inoculated intraperitoneally with 5×10^6 hybridoma cells in 0.5 ml of saline. Ascitic fluids were collected after 6 to 12 days. Lipids were removed with Freon 113, and complement was inactivated by incubation of the ascitic fluid at 56°C for 30 min, followed by precipitation in 50% ammonium sulfate. The precipitate was dissolved in saline, dialyzed against saline, and frozen in small portions at −70°C.

ELISA for anti-fimbrial antibody determination. The ELISA used in this study was a modification of the procedure described by Dodd and Eisenstein (4). Microtiter plates (activated polyvinyl chloride; Flow) were coated overnight at 30°C with 100 μl per well of a fimbria solution (2.5 μg/ml) in 0.05 M bicarbonate coating buffer (pH 9.6). The plates were washed three times with phosphate-buffered saline–0.05% Tween 20 (PBST). For hybridoma screening, the wells were incubated with 100 μl of supernatant for 2 h at room temperature. For titer determinations and specificity testing, the wells were incubated with serial dilutions of polyclonal antiserum or monoclonal antibodies (MAbs) in PBST containing 0.5% bovine serum albumin (Boehringer Mannheim Biochemicals). Subsequently, the wells were washed three times with PBST, followed by incubation with conjugate for 3 h at room temperature. In the case of MAbs, goat antimouse immunoglobulin G (IgG) (heavy and light chains) conjugated to peroxidase (Nordic Laboratories) was used, and in the case of polyclonal antiserum, goat antirabbit IgG (heavy and light chains) conjugated to peroxidase (Nordic) was used. Anti-fimbrial antibody activity was detected colorimetrically by adding 0.04% *o*-phenylenediamine in citrate-phosphate buffer (pH 4.5) to 0.005% H_2O_2. The reaction was developed in the dark for 15 min at room temperature and stopped with 2 M H_2SO_4. The A_{492} was measured in a Titertek Multiskan (Flow).

Class determination of MAbs. After microtiter plates were coated with fimbriae, homologous MAbs were added as described for the ELISA. After three washings with PBST containing 0.5% bovine serum albumin, goat antimouse IgG1, IgG2a, IgG2b, or IgM (Nordic) was added as the second antibody. MAb classes were finally detected after the addition of rabbit antigoat IgG (heavy and light chains) conjugated to peroxidase (Nordic).

Spot test for the reaction of MAbs with denatured fimbriae. Fimbriae were denatured by being boiled for 5 min in a buffer containing 0.06 M Tris (pH 6.8), 2% SDS, and 0.05% β-mercaptoethanol. Denatured fimbriae were spotted onto nitrocellulose paper (2 μg per spot) and dried overnight at room temperature. The nitrocellulose paper was blocked with 1% bovine serum albumin in PBST for 1 h at 37°C, followed by incubation for 1 h at 37°C with 1:100-diluted MAbs. After three washings with PBST, the paper was incubated with 1:100-diluted goat antimouse IgG-peroxidase conjugate in PBST containing 1% bovine serum albumin for 1 h at 37°C. The paper was washed three times with PBST, and immune complexes were detected by adding 0.05% diaminobenzide–0.002% H_2O_2 in phosphate-buffered saline. After 10 min, the reaction was stopped with 2 M H_2SO_4.

HA assays. The HA activity of human erythrocytes and the inhibition of HA were determined by the method of van den Bosch et al. (25). Briefly, serial dilutions of bacteria were incubated with human erythrocytes for 2 h on ice. HA was read after agitation. The inhibition of HA was tested with bacteria preincubated for 30 min at room temperature with polyclonal antiserum or MAbs. Antibody dilutions which did not cause agglutination of bacteria, as checked by microscopy, were used.

RESULTS

Characterization of polyclonal antisera raised against $F7_1$, $F7_2$, F9, and F11 fimbriae. All fimbriae were purified as described in Material and Methods. The purity of these fimbria preparations was checked by SDS-polyacrylamide gel electrophoresis (Fig. 1). Polyclonal antisera were raised in rabbits against purified $F7_1$, $F7_2$, F9, and F11 fimbriae. Cross-reactions of these polyclonal antisera with all purified fimbriae were analyzed in an ELISA. All polyclonal antisera reacted strongly with the homologous fimbriae (Table 2). However, all polyclonal antisera also reacted with the heterologous fimbriae, although with lower titers than in the homologous reactions. The anti-$F7_1$ antiserum reacted with all the fimbriae except the Pap fimbriae. In the case of anti-$F7_2$ antiserum, positive reactions were found with all but the 1A fimbriae. The anti-F9 antiserum reacted strongly with the F9 fimbriae and also showed a positive reaction with the $F7_1$, $F7_2$, F11, and 1A fimbriae. The anti-F11 antiserum showed the highest titers against F11 and Clegg fimbriae and also recognized antigenic determinants on the other fimbriae which cause MRHA but not on the 1A and 1C fimbriae.

MAb production. Purified $F7_1$, $F7_2$, F9, and F11 fimbriae were used for MAb production as described in Material and Methods. Three weeks after the various fusions, the hybridomas with the highest ELISA results were selected for additional growth. After subcloning, the hybridomas with the highest titers in the ELISA were used for the production of ascitic fluids, and the immunoglobulins were characterized after partial purification from these ascitic fluids. The results of the immunoglobulin subclass determinations are shown in Table 3. Six MAbs were of the IgG1 subclass, six were of the IgM subclass, three were of the IgG2b subclass, and one was of the IgG2a subclass.

Characterization of MAbs raised against $F7_1$, $F7_2$, F9, and

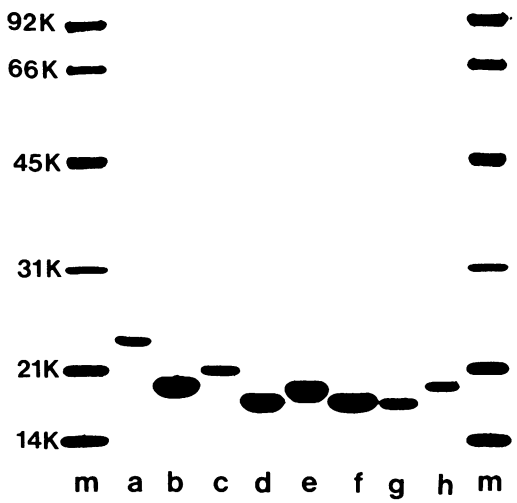

FIG. 1. SDS-polyacrylamide gel electrophoresis with different purified fimbriae. Lanes: a, $F7_1$; b, $F7_2$; c, F9; d, F11; e, Pap; f, Clegg; g, 1C; h, 1A; m, marker.

F11 fimbriae. Partially purified MAbs from ascitic fluids were tested in the ELISA for (cross-)reactions with all purified fimbriae. In Table 3, the titers are shown for each MAb against homologous and heterologous fimbriae. The four M6 MAbs raised against $F7_1$ fimbriae only recognized an epitope on $F7_1$ fimbriae. All three M4 MAbs, which were produced against F9 fimbriae, recognized only epitopes on F9 fimbriae. The M2 MAbs, raised against $F7_2$ fimbriae, could be divided into two groups: M2-7, which only recognized an epitope on $F7_2$ fimbriae, and M2-1 and M2-11, which recognized epitopes on $F7_2$ and F9 fimbriae. The M7 MAbs, raised against F11 fimbriae, could also be divided into two groups: two MAbs (M7-5 and M7-15) which recognized epitopes on F11, $F7_2$, and Pap fimbriae, and a group of four MAbs (M7-4, M7-6, M7-7, and M7-13) which recognized epitopes on F11 and Clegg fimbriae.

Purification of MAbs by high-pressure liquid chromatography yielded antibodies which still showed the same (cross)-reactions as partially purified MAbs. Since immunization and the ELISA were performed with native fimbriae, we analyzed the reaction of the MAbs with denatured fimbriae in a spot test as described in Materials and Methods. Only two sets of MAbs reacted with denatured homologous fimbriae (Fig. 2). Firstly, both the M2-1 and M2-11 MAbs reacted with denatured $F7_2$ fimbriae, and secondly, the M7-4, M7-6, M7-7, and M7-13 MAbs showed a positive reaction with denatured F11 fimbriae. These M2 and M7 MAbs also reacted with the denatured heterologous F9 and Clegg fimbriae, respectively (data not shown).

TABLE 2. Cross-reactions of polyclonal antisera in an ELISA with cloned, purified fimbriae

Fimbriae that antiserum was raised against	Antibody titer[a] to indicated fimbriae							
	$F7_1$	$F7_2$	F9	F11	Pap	Clegg	1A	1C
$F7_1$	5.0	4.1	4.1	2.9		2.0	2.6	2.0
$F7_2$	3.5	5.0	4.1	3.2	2.9	2.9		2.0
F9	2.0	3.2	4.7	2.3			2.3	
F11	3.5	3.5	3.2	4.4	3.2	4.4		

[a] Given as the logarithm of the last reciprocal dilution of the antiserum giving an A_{490} of at least 0.5.

TABLE 3. Titers of MAbs in an ELISA with eight different purified fimbriae and subclasses of antibodies

MAb	Fimbriae that MAb was raised against	MAb subclass[a]	Antibody titer[b] to indicated fimbriae					
			$F7_1$	$F7_2$	F9	F11	Pap	Clegg
M6-3	$F7_1$	IgG2a	4.4					
M6-6		IgM	3.8					
M6-8		IgM	4.1					
M6-19		IgM	4.7					
M4-5	F9	IgM			4.1			
M4-7		IgM			4.7			
M4-11		IgM			3.5			
M2-1	$F7_2$	IgG2b		5.0	3.8			
M2-11		IgG1		3.5	2.0			
M2-7		IgG1		4.4				
M7-4	F11	IgG2b				4.4		4.1
M7-6		IgG1				5.0		5.0
M7-7		IgG2b				4.1		4.1
M7-13		IgG1				4.4		4.1
M7-5		IgG1		4.1		4.1	3.8	
M7-15		IgG1		4.7		4.1	3.5	

[a] Determined as described in Materials and Methods.
[b] Given as the logarithm of the last reciprocal dilution of the antiserum giving an A_{490} of at least 0.50. 1A and 1C fimbriae, were nonreactive.

HA inhibition assays. The MRHA titer of AM1727 with different clones was tested in an HA assay. All four clones showed MRHA, and this MRHA was completely inhibited in the presence of the homologous polyclonal antiserum (Table 4). In the presence of MAbs no inhibition of MRHA was observed.

DISCUSSION

In this study, we described the production of polyclonal antisera and MAbs against the four purified P fimbriae $F7_1$, $F7_2$, F9, and F11. All fimbriae were purified from cloned strains. The main advantage of using cloned strains instead of parental strains is that cloned strains only express one type of fimbria, whereas parental strains can express different types of fimbria, e.g., C1212, which expresses $F7_1$, $F7_2$, and 1C fimbriae (28). Another advantage of using cloned

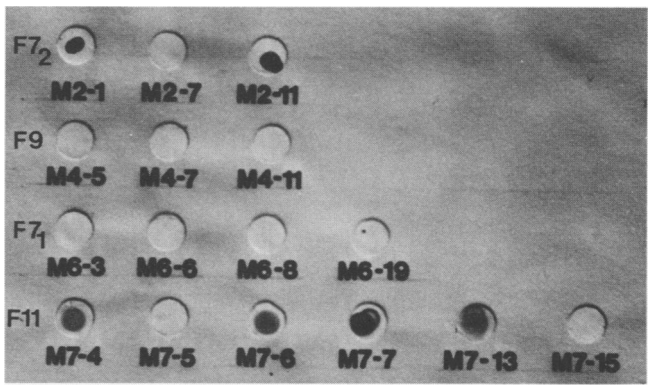

FIG. 2. Spot test with MAbs and denatured fimbriae.

TABLE 4. HA inhibition by polyclonal antisera and MAbs

Plasmid (fimbriae)	Antiserum	HA titer
pPIL110-37 ($F7_2$)	None	128
	Polyclonal	0
	M2-1	64
	M2-7	64
	M2-11	64
pPIL288-10 (F9)	None	256
	Polyclonal	0
	M4-5	128
	M4-7	128
	M4-11	128
pPIL110-70 ($F7_1$)	None	128
	Polyclonal	0
	M6-3	128
	M6-6	64
	M6-8	64
	M6-19	32
pPIL291-15 (F11)	None	128
	Polyclonal	0
	M7-4	128
	M7-5	128
	M7-6	128
	M7-7	128
	M7-13	128
	M7-15	128

strains is that these strains give rise to high fimbria production, which facilitates the purification of fimbriae. All fimbriae used in this study were pure, as showed by SDS-polyacrylamide gel electrophoresis (Fig. 1). Polyclonal antisera raised against purified fimbriae reacted with the homologous fimbriae (Table 2) but also with other fimbriae. These results are in agreement with the results of Korhonen et al. (11), who made a set of antisera raised against purified fimbriae from parental strains. These fimbria preparations did contain different fimbriae, because the parental strains possessed different types of fimbria. Our fimbria preparations only possessed only type of fimbria, and the antisera raised against these fimbriae still showed numerous cross-reactions in the ELISA (Table 2). This result indicates that these fimbriae have various antigenic determinants in common.

With the same pure fimbria preparations we produced a set of MAbs against $F7_1$, $F7_2$, F9, and F11 fimbriae. The MAbs against $F7_1$ and F9 fimbriae only recognized epitopes on $F7_1$ and F9 fimbriae, respectively. It is not yet certain whether both series of MAbs recognize the same or different epitopes within each series. Recent data from DNA sequencing of the fimbria genes coding for the Pap (1) and $F7_2$ (27) fimbriae indicate that there are several pronounced antigenic determinants on fimbriae. Thus, it might be possible that the M4 and M6 MAbs recognize different epitopes on F9 and $F7_1$ fimbriae, respectively. The M2 MAbs, raised against $F7_2$ fimbriae, could be divided into two groups: the M2-7 MAb, which only recognized an epitope on $F7_2$ fimbriae, and the M2-1 and M2-11 MAbs, which recognized epitopes on $F7_2$ and F9 fimbriae. From these results, we conclude that M2-1 and M2-11 probably recognize the same epitope and that this epitope is only present on $F7_2$ and F9 fimbriae. This conclusion was supported by the spot test with denatured fimbriae. Both M2-1 and M2-11 recognized epitopes on denatured $F7_2$ and F9 fimbriae, in contrast to M2-7, which did not react with denatured fimbriae. The M7 MAbs, raised against F11 fimbriae, could also be divided into two groups: two MAbs (M7-5 and M7-15) which recognized epitopes on F11, $F7_2$, and Pap fimbriae, and a group of four MAbs (M7-4, M7-6, M7-7, and M7-13) which recognized epitopes on F11 and Clegg fimbriae. This last group of MAbs also recognized epitopes on denatured F11 and Clegg fimbriae, as shown in the spot test, in contrast to M7-5 and M7-15, which did not react with denatured fimbriae. From these results, we conclude that M7-5 and M7-15 probably recognize the same epitope and that this epitope is present in F11, $F7_2$, and Pap fimbriae. It is likely that M7-4, M7-6, M7-7, and M7-13 also recognize a common epitope on F11 and Clegg fimbriae but that this epitope is different from the one recognized by M7-5 and M7-15.

Most of the MAbs did not react with denatured fimbriae. This is not surprising, because the mice were immunized with native fimbriae. The reaction of MAbs M2-1, M2-11, M7-4, M7-6, M7-7, and M7-13 with denatured fimbriae suggests that the epitopes recognized by these MAbs are not involved in a quaternary structure. In contrast, all polyclonal antisera tested so far have reacted strongly with denatured fimbriae (3, 3a). Furthermore, the MAbs were far more specific than the polyclonal antisera. The most likely explanation for these differences between MAbs and polyclonal antisera is that an MAb only recognizes one epitope, whereas a polyclonal antiserum recognizes many epitopes, some of which are also present in heterologous P and even type 1 fimbriae. This is supported by the finding that the combination of two different MAbs raised against F11 fimbriae (e.g., M7-4 and M7-5) already recognized four different P fimbriae.

No inhibition of MRHA by MAbs was found, in contrast to the complete inhibition by polyclonal antisera (Table 4). Recent data from Norgren et al. (16) and Lindberg et al. (13) indicated that the adhesin of Pap fimbriae is a minor component of these fimbriae. Since this is also the case for the $F7_1$, $F7_2$, F9, and F11 fimbriae (I. van Die, E. Zuidweg, W. Hoekstra, and H. Bergmans, FEMS Meeting on Molecular Biology of Microbial Pathogenicity, 17–20 June 1985, p. 18), it is not surprising that we were not able to select an MAb which inhibited MRHA. The probability of finding an MAb which recognizes an epitope involved in the adhesive properties of these fimbriae is very low when the adhesin is only a minor component of the fimbriae. Since the MAbs described in this paper are far more specific than polyclonal antisera, these MAbs may be useful for diagnostic and epidemiological means. Furthermore, the MAbs can be very helpful in studies concerning antigenic variation and phase variation of P fimbriae.

ACKNOWLEDGMENTS

This work was supported by the Dutch Kidney Foundation (grant 81.320).

We are grateful to H. A. Valkenburg (Erasmus University, Rotterdam, The Netherlands) and P. A. M. Guinee (State Institute for Public Health, Bilthoven, The Netherlands) for support and advice. We thank Kees Verstijnen and Marc de Baets (State University of Limburg, Maastricht, The Netherlands) for introducing us to the hybridoma techniques and for valuable suggestions during the experiments.

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